Trichome initiation during leaf growth in *Pelargonium scabrum*

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Statistical and scanning electron microscopical investigations were carried out to study trichome initiation during leaf development in *Pelargonium scabrum* (L.) L'Hérit. It is evident that leaf growth in *P. scabrum* can be divided into different stages of epidermal cell differentiation and enlargement, and trichome initiation. Glandular hairs are initiated continually during leaf growth, although at varying rates during the different stages of leaf development, while spiny hairs are initiated in the young leaf only. Because the rate of glandular hair initiation is lower than that of epidermal cell differentiation and enlargement, the indumentum becomes less dense with leaf expansion.

S. Afr. J. Bot. 1984, 3: 50 – 54

Statistiese en aftaselektronmikroskopiese ondersoeke is uitgevoer om trigoominisiëring tydens blaarontwikkeling by *Pelargonium scabrum* (L.) L'Hérit. te bestudeer. By *P. scabrum* kan blaargroei klaarblyklik verdeel word in verskillende stadia van epidermisseldifferensiëring en -vergroting, en trigoominisiëring. Klierhare word voortdurend tydens blaargroei geïnisieer, hoewel teen variërende tempo in die verskillende stadia van blaarontwikkeling, terwyl stekelhare slegs in die jong blaar geïnisieer word. Aangesien die tempo van klierhaarinisiëring laer is as dié van epidermisseldifferensiëring en -vergroting, word die indumentum yler tydens blaargroei.

S.-Afr. Tydskr. Plantk. 1984, 3: 50 – 54

Keywords: Leaf growth, *Pelargonium scabrum*, trichome initiation

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Accepted 14 October 1983

Introduction

The ubiquitous tendency of trichome production in the plant world suggest that the potential for the development of a hairy covering must be available in the gene pool of plants occupying essentially all terrestrial environments (Johnson 1975). Netolitzky (1932) proposes that every epidermal cell is a potential trichome initial. The expression of this potential is influenced by various factors which not only determine whether a trichome will develop or not, but also what morphological type of trichome will be formed. The type of indumentum, however, is often more influenced by the distribution and density of trichomes, than by the morphology of the individual trichomes (Johnson 1975).

Where trichome initiation continues throughout ontogeny, different trichome types may be produced at different developmental stages of an organ (Hammond & Mahlberg 1973), or the entire plant (Stober 1917). According to Turner *et al.* (1980) physiological differences between vegetative and reproductive parts in *Cannabis sativa* may be the cause of the development of different trichome types in these parts. It is also possible that the control mechanisms for the development of glandular hairs differ from those for non-glandular hairs (Turner *et al.* 1980).

In many plants the dense indumentum of the young leaves becomes sparse with leaf growth. This occurs when trichomes are initiated in young leaves only, for example in *Beyeria viscosa*, where the density of glandular hairs decreases sharply with the increase in leaf area (Dell & McComb 1974). Trichomes may also be initiated continually during leaf development, as is the case in *Cannabis sativa* (Turner *et al.* 1980) and *Newcastelia viscida* (Dell & McComb 1975). The change in trichome density will then depend upon the rate of trichome initiation during leaf growth. Where the rate of leaf growth exceeds that of trichome initiation, the indumentum will become sparser despite a continual production of trichomes. Trichome initiation may, however, also keep pace with the expanding leaf surface, in which case the density of the indumentum will remain unchanged (Johnson 1975).

Pelargonium L'Hérit. is well known for its commercial value in the perfume industry. Cultivars of various *Pelargonium* species are grown for distillation of the so-called 'geranium oil' (Schery 1972; Gulati *et al.* 1977) which is produced in glandular hairs (Fahn 1979). The variation in trichome density during different stages of leaf development is, however, unknown. The aim of this research was to determine whether trichome initiation occurs once only or continually during leaf development; whether the trichome density decreases with leaf growth;

and whether there is variation in the rate of trichome initiation relative to that of leaf expansion. The work was carried out on *Pelargonium scabrum* (L.) L'Hérit., as part of the study on trichomes and biosynthesis of essential oils in *Pelargonium*.

Material and Methods

The specimens studied were prepared from cuttings of the same mother plant used in the previous investigation of the trichomes of *P. scabrum* (Oosthuizen 1983). The uppermost three leaves of five stems, or six leaves of one stem, were investigated during springtime (October) after the plants had been growing under natural environmental conditions throughout the year.

Counting of trichomes and epidermal cells

Trichomes and epidermal cells were counted ad- and abaxially on the lamina. Because the indumentum is too dense and the trichomes are too small to be counted under 30× magnification, the dissecting microscope could not be used in this investigation. Fresh leaves were therefore affixed to microscope slides and trichomes were counted with the aid of a Zeiss light microscope, a fibre optic lamp being used as the light source.

Glandular hairs were counted in microscope fields scattered over the leaf surface. The leaf base and apex were, however, avoided since the trichome density varies considerably in these parts. Owing to the density of the indumentum in young leaves the glandular heads were counted with the aid of a *camera lucida* attachment. Glandular heads were counted in separate fields until a 96% probability was reached that the deviation from the mean was less than 10%. The following formula was used:

$$\text{Number of fields} = \left[\frac{1{,}96 \times \text{standard deviation}}{10\% \times \text{mean}}\right]^2$$

Owing to the sparse distribution of the spiny hairs, their density was ascertained on the whole leaf surface, excepting the leaf margin. The leaf area was measured in cm^2 with a LI-COR-3000 apparatus and impressions of the ad- and abaxial surfaces were subsequently made with colourless nail varnish. These replicas were mounted in water on microscope slides and the epidermal cells were counted in the same way as described for the glandular hairs with the exception that transmission light was used.

The area of the microscope field was calculated for the different magnifications used in the various countings and the density of the trichomes and epidermal cells were expressed as 'number per mm^2'. All the available data were represented graphically with a Hewlett Packard 7210A two-dimensional plotter.

Scanning electron microscopy

The variation in trichome density with leaf age was also observed with the scanning electron microscope. The six uppermost leaves of four stems were fixed at room temperature for 24 h in 3% glutaraldehyde (Merck) in 0,05 mol dm^{-3} phosphate buffer, pH 7,2 and subsequently washed in a solution of the same buffer in 0,34 mol dm^{-3} sucrose and dehydrated in a graded acetone series. Specimens were subsequently critical point dried with liquid CO_2, gold sputtered and viewed with a Jeol JSM-35 scanning electron microscope at 12 kV.

Discussion of Results

The indumentum of the lamina of *P. scabrum* consists of only two trichome types, i.e. five-celled glandular hairs and unicellular spiny hairs (Oosthuizen 1983). The glandular hairs occur on the entire leaf surface whereas the development of spiny hairs is restricted almost exclusively to areas of the veins and leaf margin. The denser indumentum at the base than at the apex of the leaf primordium suggests that trichomes are initiated acropetally from the leaf base to the leaf apex (Figure 1.1).

The scanning electron microscopical investigation of the trichome density in leaves of varying age revealed that in the leaf primordium spiny hairs are apparently initiated before glandular hairs; in the young leaf the spiny hairs are already mature and extremely conspicuous in comparison to the glandular hairs which are predominantly young (Figure 1.1). The initiation of spiny hairs, however, either comes to an end at an early stage of leaf development or continues at a very low rate. The spiny hairs thus become more sparsely distributed on the expanding lamina whereas the glandular hairs become more conspicuous and do not decrease in density to the same extent (Figures 1.2 – 1.4). This implies that glandular hair initiation continues during leaf development and that the rate of initiation exceeds that of spiny hair initiation but is lower than the rate of leaf expansion. This observation was also confirmed statistically, as well as the fact that the continual initiation of glandular hairs occurs at varying rates during the different stages of leaf development (Figure 2.2). The continual initiation of glandular hairs implies a continual increase in the total number of trichomes present in the indumentum and clarifies the observation that glandular hairs of various morphological types representing different developmental stages of a single trichome type (Oosthuizen 1983), occur on a single leaf and are present on young and mature leaves. The oldest leaves are often smaller than those directly above them. From a certain position, however, the leaf area decreases towards the stem apex. This can be caused by various factors: (i) there is a lack of assimilates in the young plant, (ii) the oldest leaves develop while the young stem apex, and therefore also the leaf primordia, are small and (iii) the oldest leaves developed under less favourable environmental conditions than the younger ones, which were initiated in spring. A graphic representation, however, revealed the existence of a linear correlation between leaf area, length and width in leaves of all ages and sizes.

Despite quantitative differences between the indumentum and epidermal cells, ad- and abaxially, there is a linear correlation between ad- and abaxial values. A graph of leaf size versus glandular hair density, or versus the total number of glandular hairs, thus shows the same tendency for the adaxial values as for the abaxial values. In this study, where absolute values are not considered but only tendencies and relative values, graphic representations of adaxial and abaxial values could therefore be used interchangeably.

A linear correlation also exists between the glandular hair and epidermal cell densities. This suggests that certain epidermal cells are probably predestined to develop into trichomes. Further investigation will be carried out to ascertain possible factors that can influence the appearance of an epidermal cell as a glandular hair initial. Whether these factors are genetically fixed or not, will also be dealt with after further study of specimens cultivated under various environmental conditions.

The graphic representation of leaf area versus epidermal cell density shows the same tendency as that of area versus glandular hair density (Figure 3), hence the existence of a linear relationship between epidermal cell and glandular hair density. Initially there is an exponential decrease in glandular hair and epidermal cell density with leaf expansion, followed by a gradual decrease until the density remains almost constant.

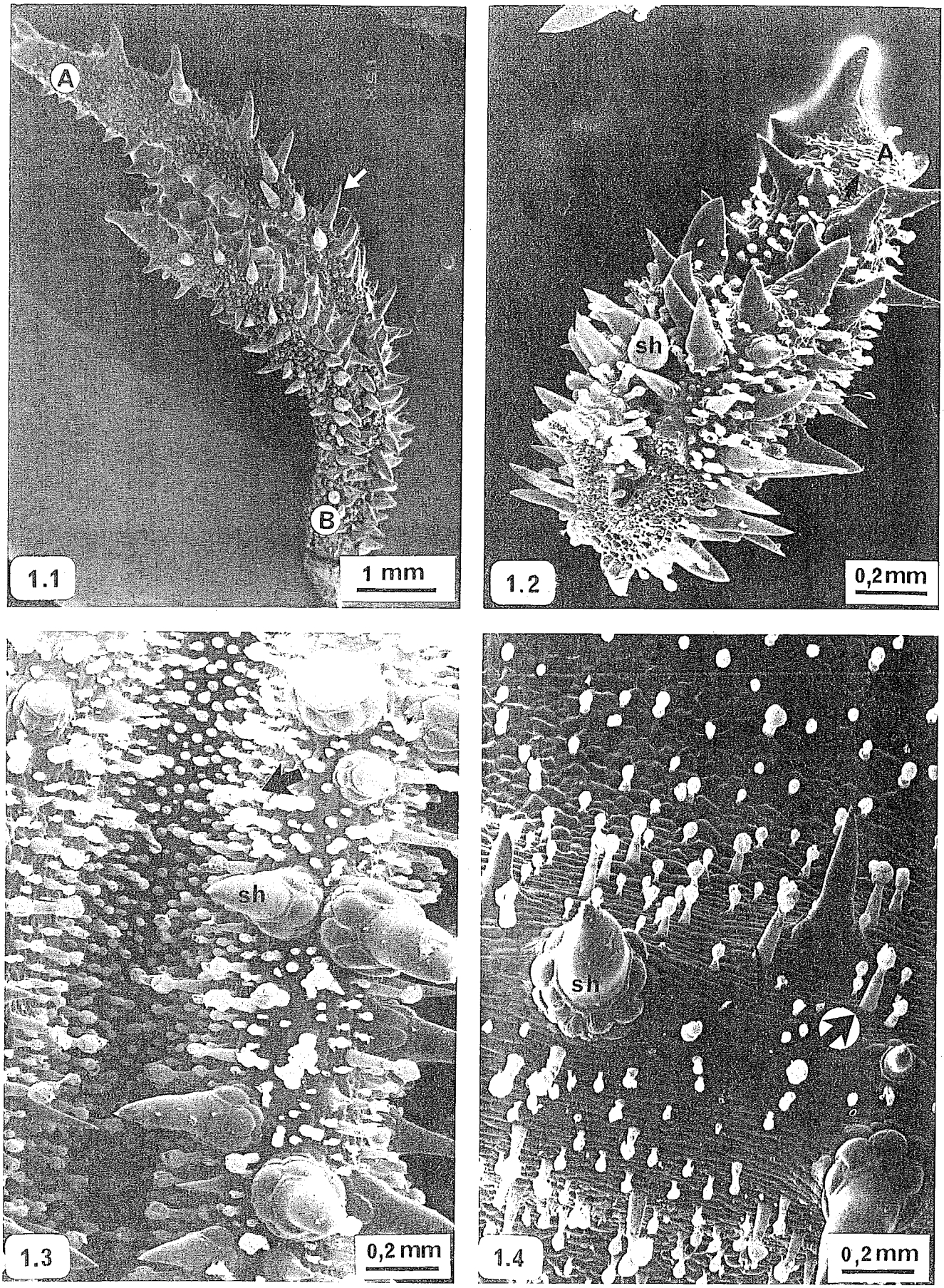

Figure 1 Scanning electron micrographs of the indumentum of the lamina of *P. scabrum* at different stages of leaf growth. 1.1: Young leaf of *P. scabrum* with conspicuous spiny hairs (arrow) and denser indumentum at the base (B) than at the apex (A) of the leaf. 1.2 – 1.4: Consecutive leaves from the stem apex; micrographs with the same magnification to illustrate the change in the indumentum density with leaf growth. 1.2: Young leaf of *P. scabrum*. Spiny hairs (sh) form most conspicuous part of indumentum, glandular hairs (arrow) are sparsely distributed at leaf apex (A). 1.3: Relatively young leaf of *P. scabrum* (older than in Figure 1.2). The indumentum is dense with glandular hairs (arrow) forming the most conspicuous part and spiny hairs (sh) occurring only on the veins. 1.4: Mature leaf of *P. scabrum*. Indumentum is relatively sparse with glandular hairs (arrow) occurring on the entire surface of the lamina and spiny hairs (sh) only on the veins.

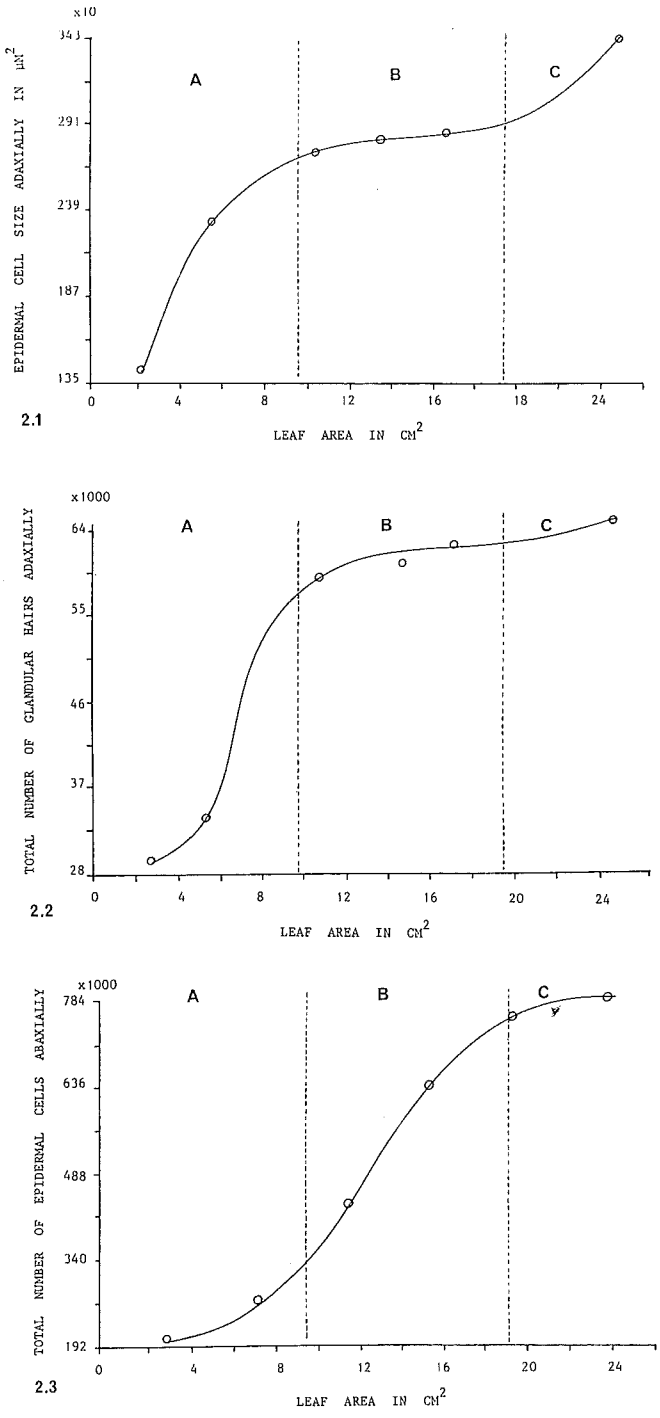

Figure 2 Graphic representation of leaf area versus (2.1) cell sizes of adaxial epidermis, (2.2) total number of adaxial glandular hairs and (2.3) total number of abaxial epidermal cells. Data were obtained from the six uppermost leaves of one branch of *P. scabrum*. The same tendency is shown as for data obtained from the opposite surface of the lamina (ad- or abaxial). A = phase with relatively rapid rate of initiation of glandular hairs (2.2) and differentiation of epidermal cells (2.3), as well as enlargement of epidermal cells (2.1). B = phase with rapid epidermal cell differentiation (2.3) but almost no glandular hair initiation (2.2) or epidermal cell enlargement (2.1). C = phase with gradual hair initiation (2.2) and epidermal cell enlargement (2.1) but almost no epidermal cell differentiation (2.3).

In the latter stage glandular hair initiation occurs at the replacing rate; the rates of glandular hair initiation and epidermal cell differentiation are thus identical.

The exponential decrease in the density of glandular hairs and epidermal cells (Figure 3) coincides with an exponential increase in the size of the epidermal cells (Figure 2.1:A), as

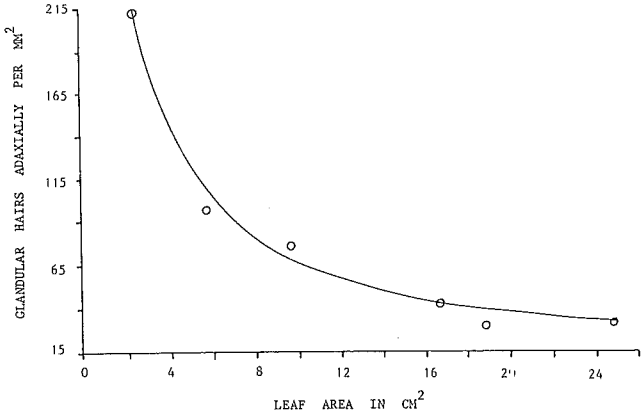

Figure 3 Graphic representation of leaf area versus adaxial glandular hair density. Leaf expansion is attended with an initial exponential decrease in density. In the mature leaf the density remains almost constant. Data were obtained from the three uppermost leaves of five branches of the same plant of *P. scabrum*. The same tendency is shown for abaxial values, as well as for the graphs of leaf area versus epidermal cell density.

well as with an exponential increase in the total number of glandular hairs and epidermal cells (Figures 2.2:A & 2.3:A). The leaf surface thus also expands rapidly.

The initial phase of exponential increase in the size of the epidermal cells and the total number of glandular hairs is followed by a phase where the curve flattens and the size of the epidermal cells and the total number of glandular hairs remain almost constant. This coincides with a rapid increase in the total number of epidermal cells (Figures 2.1:B, 2.2:B & 2.3:B). In the following phase, where the size of the epidermal cells and the total number of glandular hairs increase gradually, the rate of increase in the total number of epidermal cells slows down (Figures 2.1:C, 2.2:C & 2.3:C). Consequently the leaf expands more slowly.

The similarity between the graphic representations of epidermal cell size and the total number of glandular hairs (Figures 2.1 & 2.3) suggests that the total number of glandular hairs is correlated with the size of the epidermal cells and that glandular hair initiation probably coincides with epidermal cell enlargement. Glandular hairs are, however, probably still initiated in epidermal cells predestined to function as glandular hair initials.

The development of spiny hairs shows the same tendency as that of glandular hairs, where an exponential decrease in density occurs with leaf expansion. The drastic decrease in the ratio of spiny hairs to glandular hairs (Figure 4), however, suggests that spiny hairs are either initiated at a much lower rate than glandular hairs or are initiated only during the primordial stages of leaf development, with no further spiny hairs developing during leaf expansion.

Conclusions

Interpretation of data is complicated throughout by the complex system of dynamic developmental features occurring simultaneously. While there is a decrease in density, initiation of glandular hairs and differentiation of epidermal cells continues, with a consequent increase in their total number. Existing epidermal cells enlarge, some develop into glandular hairs, and the whole leaf expands as a result of the differentiation and enlargement of epidermal cells. The relative rates of the various processes are not necessarily identical and the different phases of every process also occur at different rates.

Simultaneous interpretation of the graphic representations

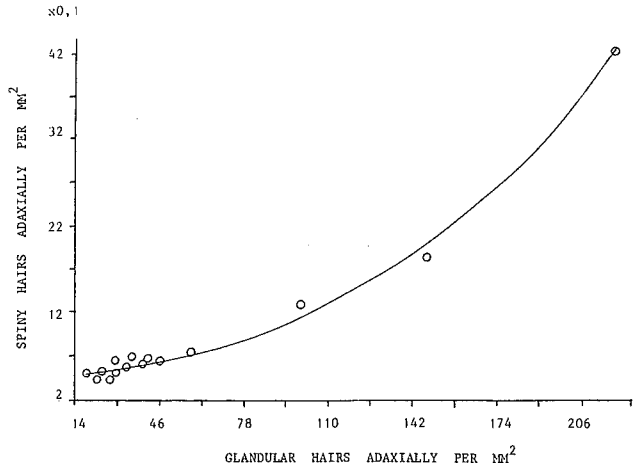

Figure 4 Graphic representation of adaxial glandular hair density versus adaxial spiny hair density. An exponential decrease in the ratio of spiny hairs to glandular hairs occurs during leaf growth. Young leaves have a dense indumentum of glandular and spiny hairs but in the mature leaf spiny hairs are much more sparsely distributed than glandular hairs. Data were obtained from the three uppermost leaves of five branches of the same plant of *P. scabrum*. The same tendency is shown for abaxial values.

of leaf size versus glandular hair and epidermal cell density, epidermal cell size, total number of glandular hairs and total number of epidermal cells (Figures 2 & 3) led to the conclusion that leaf growth can be divided into different phases of epidermal cell differentiation and enlargement and trichome development. According to the above-mentioned graphs cell enlargement coincides with glandular hair initiation, in a phase of relatively rapid epidermal cell differentiation, and consequently also rapid leaf expansion (Figures 2.1:A, 2.2:A & 2.3:A). When epidermal cell differentiation subsequently increases, epidermal cell enlargement and glandular hair initiation almost cease (Figures 2.1:B, 2.2:B & 2.3:B). During the subsequent increase in the rate of epidermal cell enlargement and glandular hair initiation, there is almost no epidermal cell differentiation (Figures 2.1:C, 2.2:C & 2.3:C) and the leaf therefore also expands very little.

It is thus evident that glandular hair initiation is a process occurring continually during the development of the leaf of *P. scabrum*. Owing to the low rate of glandular hair initiation in comparison to epidermal cell differentiation and enlargement (and thus leaf expansion), the density of the indumentum, however, decreases with leaf growth. Definite variation occurs in the rate of trichome initiation relative to that of leaf expansion.

Acknowledgements

The Electron Microscope Unit of the University of Stellenbosch is thanked for assistance, the Department of Wood Science, University of Stellenbosch, for the use of the Hewlett Packard plotter and the C.S.I.R. for financial support.

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